*Review Article*

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# A review of the applications of bioproteins in the preparation of biodegradable films and polymers

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ABSTRACT

Environmental pollution due to the entry of synthetic polymers, plastics and non-degradable packaging materials into nature is one of the greatest dangers that threaten human life. Therefore, in recent years, the use of dietary proteins in the production of biodegradable films has intensified. Protein-based films have attracted a lot of attention because of their advantages, including their ability to be used in a variety of materials packaging. Protein films are used for small food packaging, especially special products such as beans, fruit kernels and cashews. Protein films can also be used to prevent spoilage and moisture migration in foods such as pizza, staples and candies. Protein films have good resistance to the passage of oxygen gas at low relative humidity and have good mechanical properties and turbidity. These films, like synthetic polymers, are used to package food, especially products that are individually packaged, such as beans, nuts, and peanuts. These films can be modified with antimicrobial and antioxidant agents to form bioactive films. Proteins such as corn husk, glutenin and gladin, soy protein, gelatin, collagen, meat myofibril, milk casein, milk whey protein and egg protein are widely used in the production of biodegradable polymers. In this review article, we have tried to study the properties of protein polymers and their applications.

## 1. Introduction

Petroleum polymer films are widely used in the packaging industry due to their easy formability, cheap price, lightness, and high chemical resistance, variety of physical properties, heat-sealing capability, good printability and easy production process. In addition to their many benefits, these materials also have limitations. Pollution from these polymers such as burial, incineration and recycling has attracted the attention of researchers in recent years to using natural biodegradable polymers on the production of packaging materials [1, 2]. Biodegradable polymers not only consume less energy in the manufacturing process than existing plastics, but are also of particular importance because of their renewable consumables. The term biodegradable refers to the ability to break down the chemical structure of a substance into simpler substances such as carbon dioxide, water, methane, and biomass, which is due to the enzymatic activity of microorganisms [3, 4]. Biodegradable polymers due to the bonding of biodegradable functional groups such as ester and amide bonds in their chemical structure, after some time due to degradation processes, which are mainly aqueous or enzymatic hydrolysis, turn into shorter and water-soluble polymers in water [5]. Environmentally friendly biopolymers are a good alternative to non-renewable and non-renewable packaging materials. In other words, biodegradable films are bio-polymeric materials that are prepared from natural and renewable sources and easily are decomposed by the metabolism of living organisms after consumption under suitable conditions of humidity, temperature, and presence of oxygen. They decompose naturally and no toxic or harmful substances remain in the environment [6]. Research on biopolymers suggests biodegradable alternatives to petroleum polymers. These polymers contain a variety of polysaccharides, proteins, fats and their combinations. Protein based polymers have been considered as suitable packaging materials with high potential and carriers for antimicrobial compounds [7-9]. Fig. 1 shows the degradation of biodegradable polymers during 80 days. Due to the renewable resources used in the production of these materials, the carbon dioxide produced during the destruction by plants is converted back into raw materials. This gas is consumed after a limited time by bacteria in the environment. Finally, these

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plastics do not remain in the form of waste piles in nature. In addition, a number of biodegradable biopolymers as composted can be used in the industrial composting process. Thus, about 90% of these substances decompose in 6 months [10].


**Fig. 1.** Biodegradable polymer degradation

## 2. Types of biodegradable polymers

### *2.1. Biopolymers categories based on their origin and production process*

#### *2.1.1. Biopolymers categories based on their origin*

Biopolymers are divided into three categories based on their origin. These three categories are 1- Natural biopolymers or polymers obtained directly from biomass extraction, 2- plant carbohydrates (starch, cellulose, chitosan, alginate, agar and carrageenan) and 3- plant and animal proteins (soy protein, corn husk, wheat gluten, gelatin, Casein and whey protein) [11].

#### *2.1.2. Biopolymers categories based on production process*

Biopolymers are divided into two categories based on their production process. These two categories are 1- synthetic biodegradable polymers that are produced from biodegradable monomers using chemical processes. These include polyvinyl alcohol (PVA), polyglycolic acid (PGA), polycaprolactone (PCL), polylactic acid (PLA), and polybutylene succinate (PBS). 2- Biopolymers produced by microbial fermentation are polymers obtained by microorganisms and genetically modified bacteria. Substances such as polyhydroxy alkanoate (PHAs), polyhydroxybutyrate (PHB), bacterial celluloses, and microbial polysaccharides such as xanthan and plolan fall into this category [12]. It should be noted that not all biopolymers are not biodegradable (such as bio-based polyethenes). On the other hand, there are also polymers such as polycaprolactone that are based on fossil fuels and are biodegradable. Fig. 2 shows biopolymers categories based on their origin and production process. Fig. 3 showas biodegradability properties of renewable and fossil resources.

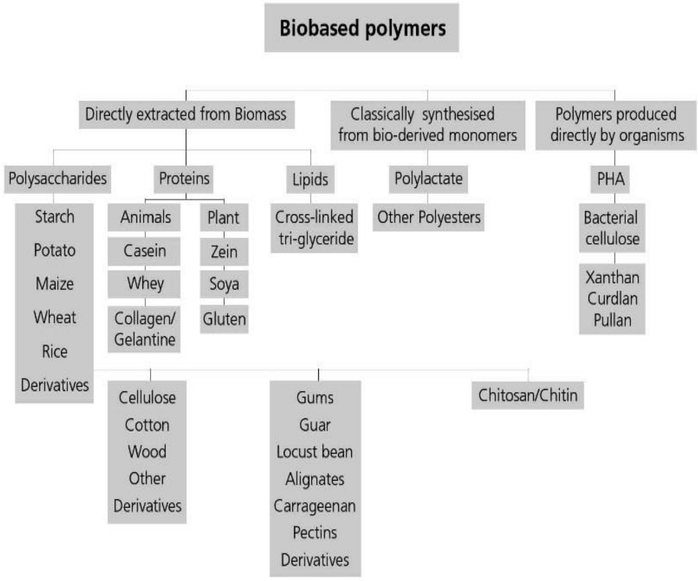


**Fig. 2.** Biopolymers categories based on their origin and production process

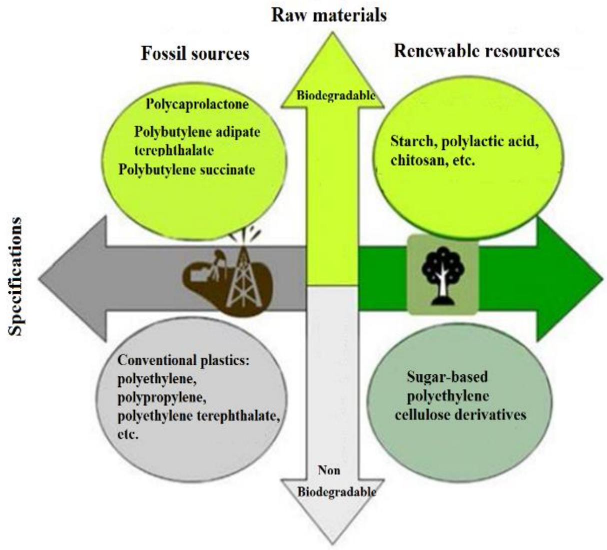


**Fig. 3.** Biodegradability properties of renewable and fossil resources

### *2.2. Types of biodegradable polymers based on nature*

Biopolymers used in packaging can be divided into four categories based on their chemical structure: proteins, polysaccharides, lipids and polyesters. 1- Proteins: such as corn germ, wheat glutenin and gladin, soy proteins, gelatin, collagen, meat myofibrils, milk casein, whey proteins and egg proteins. 2- Carbohydrates: such as cellulose and cellulose derivatives (methylcellulose, carboxymethylcellulose, and hydroxypropyl cellulose), starch and its derivatives, pectin compounds, chitin and chitosan, gums such as alginate, carrageenan, pololan, xanthan, carob and guar. 3- Lipids: animal fats and oils, wax (such as beeswax), glyceride derivatives such as glycerol and monostearate and surfactants (emulsifiers). Due to their non-polymeric structure, lipid materials do not produce continuous and independent films. These materials are used to cover the surface of medicine or food due to the desired gloss and reduced permeability. Lipids are used in combination to inhibit moisture penetration into the film structure. Composite films are in two forms. The lipid layer is placed as a sub-layer on the protein or polysaccharide layer. Lipids are dispersed in the protein or polysaccharide matrix (emulsion film). 4- Polyesters: such as polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV), polylactic acid (PLA) and polyglycolic acid. These categories are mostly non-edible biodegradable [13-15].

## 3. Advantages and disadvantages of biodegradable polymers

The most important advantage of biodegradable films and coatings over synthetic polymers is that they can act as carriers for various additives and compounds such as antimicrobials, antioxidants, etc., in which case they are called active packaging. Active packaging is a type of packaging that, in addition to having the main inhibitory properties of conventional packaging (such as gas and vapor barrier properties and mechanical stresses), improves the safety, shelf life or sensory properties of the food by changing the packaging conditions and at the same time the quality of the food is preserved [16, 17].

However, the use of biopolymers as food packaging material has various limitations that limit the use of these films. Fragility, thermal instability, low melting resistance, difficult heat sealability, high water vapor and oxygen permeability are limiting factors in the use of these films for food packaging applications. Due to the hydrophilic nature of biopolymers such as starch and cellulose, materials based on these compounds have poor water vapor barrier properties. These properties limits the long-term stability and weakens the mechanical properties of biopolymer films (sensitivity to moisture content) [18]. Other disadvantages of these films are poor processability, brittleness and vulnerability to decomposition. Finally, brittleness, stiffness, low impact resistance, and thermal instability are factors that limit the use of biopolymers as packaging materials. In general, protein-based films at moderate relative humidity are suitable barriers to oxygen gas and have desirable mechanical properties. But their barrier properties for water vapor are weak due to their hydrophilic nature [19]. Therefore, extensive research has been done to improve the performance of protein-based biopolymers. The following describes methods for improving the properties of protein-based biopolymers.

## 4. Protein based polymers

Proteins are a group of large molecules that are also polymers, made up of a sequence of more or less identical units called amino acids (protein monomers) that make up a very wide range of materials. Proteins can be thought of as alphabets and sentences due to their polymeric structure, because the alphabet with 32 letters, creates countless words. Proteins also have 20 types of monomers, and by changing their order and number, thousands of proteins are made. The way amino acids are joined together is that two monomers are placed next to each other, one of which gives H+ and the other OH-. Fig. 4 shows the general structure of amino acids, all of which differ in section R [20].

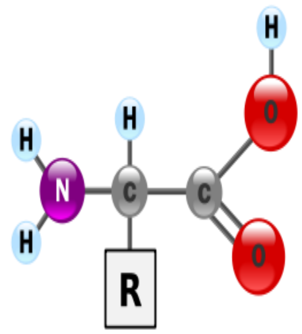


**Fig. 4.** General structure of amino acids

### *4.1. Properties of protein films*

Protein films have good resistance to the passage of oxygen gas at low relative humidity and have good mechanical properties and turbidity. However, these films are sensitive to moisture and cannot withstand water vapor. These films, like synthetic polymers [21], are used to package food, especially products that are individually packaged for scientific reasons, such as beans, nuts, and peanuts, or to be placed on top of food ingredients. They are also used in foods such as pizza, cakes and pastries to prevent moisture loss. These films can also be combined with antimicrobial and antioxidant agents [22, 23].

### *4.2. Factors affecting the properties of protein films*

#### *4.2.1. Raw material*

Protein raw materials are divided into two categories, hydrophilic and hydrophobic, to make a film based on their solubility. Hydrophilic substances such as soy protein isolate, whey protein, fish protein, mung bean protein and hydrophobic substances such as corn husk that dissolve in non-polar liquids such as alcohol. Differences in the solubility properties of raw materials affect the amount of energy required to dry the film and the way they are used in food [24].

#### *4.2.2. Polymer Chemistry*

Molecules with a more regular structure are more permeable than molecules with an irregular chemical structure, branched molecules are more cohesive than molecules without bifurcation, and molecules with lower molecular weight are more cohesive, which changes with temperature. In high-polarity polymers such as proteins and cellulose, flexibility is low due to the intramolecular forces holding the polymer chain. The desirable molecular properties for forming a protein film are asfollows. 1- The larger the molecule, the more interfacial interactions are formed, resulting in a stronger film. 2- Bipolar molecules creat unbalanced charge distribution and improve interfacial interactions. 3- Residues of polar groups can improve hydrophilicity [25].

*4.2.3. pH*

Film pH plays an important role in protein films made from water-soluble materials, such as soy protein isolate and whey protein isolate, the solubility of which depends on their isoelectric point. During dissolution, the cohesive forces between the soluble macromolecules are neutralized by units of solvent molecules. The higher the charge on the chain, the higher the interaction between the charged polymer molecules and the solvent polar molecules. The performance of the polymer is related to the properties of the solution, which in turn affects the properties of the film. The solubility of proteins increases as they move away from the isoelectric pH. But to produce an edible film at the right pH, sensory properties must also be considered along with other film properties. The films that form near the isoelectric point of the major proteins are denser and stronger [26, 27].

*4.2.4. Drying temperature*

Protein films are usually prepared using the casting method. This method involves drying a suitable colloidal protein solution, solvent and plasticizer. The effect of the drying temperature depends on the various properties of the raw material, such as the formation of a gel before drying or the formation of a thermal gel during the drying of the film. In addition, during the drying of the film, various phenomena such as: transition from the rubber phase to the glass phase, phase separation (thermodynamic incompatibility) or crystallization may occur. Heat is the most important factor in denaturing proteins. Thermal stability and deformation of proteins depend on the composition of its amino acids. During the drying of the protein, the shape of the proteins changes as the water gradually evaporates. In addition, the degree of protein folding determines the type and ratio of covalent (disulfide) or non-covalent bonds (hydrophobic, ionic, and hydrogen interactions) between protein chains [28]. The more denatured the proteins, the stronger and easier the chains can interact, especially through disulfide bonds. The cohesiveness of the final network and the performance of these links determine the characteristics of the film. As the temperature rises, the hydrophilic interactions increase, the hydrogen bonding and the electrostatic interaction decrease, and the adhesion between the polymer films and the substrate facilitates. At high temperatures (70-100 ° C) due to denaturation of proteins, harder structures are formed in protein solutions. Excessive heat or excessive evaporation rate of the solvent during drying produces discontinuous films. Water-soluble proteins (such as soy protein and whey protein) require higher temperatures and longer film formation time than alcohol-soluble proteins (such as corn husk or wheat gluten) [29].

*4.2.5. Concentration*

The concentration of the film solutions affects the adhesion of the polymers, the formation of the protein matrix and the rate of matrix formation during film preparation. At lower protein concentrations, protein-protein interactions are less likely. At the optimum concentration, the resulting film has the highest tensile strength. The optimum concentration for different protein films varies. A relatively high concentration of protein (> 8%) is required to produce whey protein isolate films in order to form disulfide bridges. While to prepare fish muscle protein film with a concentration (1.5-2%) stronger films are formed than other concentrations [30].

*4.2.6. Relative humidity*

The interaction of water with food films changes its physical properties. Absorption of water vapor by desiccants generally involves the binding of water molecules to specific hydrophilic sites, such as carboxylic, amine, and hydroxyl residues. At high relative humidity, multimolecular adsorption occurs through swelling and changes in the spatial structure of macromolecules. The relationship between equilibrium relative humidity and film moisture content can be assessed by measuring water absorption isotherms. As the relative humidity increases, the fracture force, modulus of elasticity and water vapor barrier properties decrease and the deformation at the breaking point increases [31].

*4.2.7. Film additives*

Different materials can be used to influence the mechanical, sensory, nutritional and protective properties of the protein film. Plasticizers are low molecular weight non-volatile compounds that are widely used in the polymer industry. Adding plasticizer to protein films reduces the intermolecular attraction of adjacent polymer chains and makes the film structure more flexible and soft. Plasticizers commonly used in protein films include mono-, di-, and oligosaccharides (eg, glucose or fructose syrup, honey), polyols (glycerol, polyethylene glycol, and sorbitol), fats and their derivatives (acids, stoglycerides, phospholipids and other emulsifiers). The molecular size, configuration and total number of functional groups in the plasticizer as well as its compatibility with the polymer affect the interactions between the plasticizer and the polymer [31, 32].

## 5. Methods for improving the properties of protein films

*5.1. Chemical treatment*

Proteins are biopolymers whose films are good barriers to gas. However, these films, like other biopolymers, have moderate mechanical strength and poor vapor barrier. In order to improve these features, increasing cross-linking between polymer chains is the most appropriate method. Protein networks have the ability to communicate with a wide range of active compounds [33, 34]. Chemical treatment with acid, alkali or crosslinking agents is widely used to improve the properties of the film. Theoretically, the interaction of the protein with the chemical treatment increases the chain structure, the permeability and tensile strength. Cross-linkers are sometimes used to improve the properties of protein films. These materials increase mechanical strength, reduce permeability to gases and water vapor by increasing cross-links between the chains of macromolecules.

Depending on the type of biopolymer, different compounds such as calcium, epichlorohydrin, acetic acid, tannic acid and lactic acid are used. For example, calcium is used to improve the properties of casein films. Glutaraldehyde is the most common crosslinking agent used to strengthen the structure of protein and polysaccharide wraps. Crosslinks are formed between the aldehyde group of this compound and the charged groups of biopolymers (such as amines) in proteins. However, the use of this compound and other aldehyde compounds in the production of films for the reasons mentioned should be done with caution [35, 36].

*5.2. Enzymatic treatment*

One of the effective methods to improve the inhibitory properties and mechanical strength of protein edible films is the enzymatic method. Enzymes such as transglutaminase (EC.2.3.2.13 TGase), lipoxygenase, lysine oxidase, polyphenol oxidase and peroxidase are used as cross-linkers of proteins. Transglutaminase is an enzyme that can catalyze the covalent bonds of proteins to form high molecular weight biopolymers. In general, transglutaminase improves the tensile strength of protein films, while reducing the percentage of elongation and solubility. In some cases, such as soy protein isolate films and extruded gluten films, transglutaminase treatment significantly increases the surface hydrophobicity of the films. Improving the properties of protein films using enzymes also depends on the type of protein substrate and some processing parameters, such as the amount of enzyme used [37, 38].

*5.3. Radiation treatment*

Radiation causes changes in the spatial structure of proteins, oxidation of amino acids, rupture of covalent bonds and the formation of protein free radicals, and then proteins can be converted into higher-grained granules by crosslinking, hydrophobic and electrostatic interactions, and disulfide bonding. For example, the superoxide and hydroxyl anion radicals generated by radiation in the film-forming solution modify the properties of the protein molecule. This can be due to the change of protein films by covalent bonds formed in the protein solution after irradiation [39].

*5.4.Treatment with hydrophobic materials*

As mentioned, protein films are a weak barrier to moisture. However, lipid films are a good barrier to moisture. But they are opaque, relatively inflexible, and unstable and have a waxy taste. Thus, high performance films are obtained in multi-component systems in which proteins form a continuous, cohesive network and fats creat good moisture-inhibitory properties. Lipids can form a single layer in a hydrocolloid matrix (multilayer films) or can be dispersed into the matrix (emulsion films). Addition of lipids to protein films may interfere with chain reactions and improve film flexibility. Due to the lack of structural integrity of lipids, they affect the mechanical properties of protein films [40].

**6- Important proteins used in the preparation of biodegradable polymers**

*6.1. Wheat gluten*

Wheat gluten is a by-product of starch factories and ethanol production as biofuels and is available in abundance and cheaply due to increasing demand for biofuels. Wheat gluten, when plasticized, is unique in its ability to form viscous mixtures with viscoelastic properties among cereals and other plant proteins. Glutenin and gliadin are the main constituents of wheat flour. Gliadin and glutenin are very different in molecular weight and structure. Gliadin is a monomer and glutenin is a polymer. Glutenin with a molecular weight of approximately 500×103 to 500×106 g per molecular weight is much larger than gliadin and is soluble in diluted acid while gliadin has a molecular weight of 30×103 to 80×103 g/mol and is soluble in ethanol 70% [41].

*6.1.1. Properties of wheat gluten films*

Films made from gluten are pure and transparent, so that the higher the purity, the stronger and clearer a film with mechanical properties is produced. But commercial gluten produces a matte film due to the gelatinization of the available starch. Gliadin films are clear and elastic, but glutenin films are brittle and inelastic. Gluten films are a good barrier against oxygen and carbon dioxide, but the tensile strength of this gluten film is lower than other films, and the biggest obstacle to the use of these films is their high water permeability. The tensile strength of this film is increased by adding cross-link-agents [42]. Gluten is used to coat peanuts and to encapsulate dyes and flavorings. Microencapsulation of unsaturated fatty acids by spray-dried gliadin powder reduces oxidation and produces the lowest amount of peroxide compared to other samples. Gluten-based biodegradable films, while are flexible, have sufficient strength and are also relatively transparent. Films at low relative humidity have excellent barriers to oxygen and carbon dioxide, but their low water vapor barrier and poor mechanical properties limit their use compared to synthetic films. Gluten film can also act as a carrier of additives such as antioxidants, antimicrobials and flavorings and help maintain food quality [43].

*6.1.2. Factors affecting the characteristics of gluten-based films*

Attempts to improve the properties of biopolymer films have been made by different researchers in different ways. Changes are made either as a pretreatment, ie changes in the solution that makes up the film, or by using treatments on the film. Here are some of the factors that affect production:

*6.1.2.1. Plasticizers*

Protein films are brittle by themselves. The most common way to improve film-forming properties is to add a plasticizer. Plasticizers are low molecular weight hydrophilic liquids that reduce bonding between chains, resulting in increased mobility of polymer chains, lower glass transition temperatures, and more flexible and softer films. Increased film flexibility is also associated with increased film permeability, which depends on the type and amount of plasticizer. Also, the molecular weight, concentration and properties of hydrophilic or hydrophobic plasticizer affect the migration of plasticizer from the field during storage, so for balance between optimal mechanical properties and inhibition, the use of plasticizer should be optimized [44]. Glycerol is the most widely used plasticizer.

Glycerol may be substituted with a higher molecular weight compound containing hydrophobic exchange groups that help reduce water vapor permeability and plasticizer migration, thereby increasing maximum traction before rupture in gluten films.

The substances that can be replaced with glycerol are amphiphilic substances, which include fatty acids (lauric, stearic and oleic acid), palmitic acid and octanoic acid, dibutyl phthalate and tartrate. Previous studies have been shown that with increasing temperature of process can reduce the amount of plasticizer needed to achieve the desired mechanical properties [45].

*6.1.2.2. Modification by chemical method*

Chemical treatment with acid, alkali or crosslinking agents is widely used to improve the properties of films. Wheat gluten film does not form in the isoelectric region of proteins (pH = 7-8). Adjusting the pH to acidic or alkaline conditions (below or above pH = 7-8) will contribute to the solubility of the protein and the film formation process.The film composed of alkaline solutions is more homogeneous and smooth than the films formed in the acidic solution. In alkaline environment, it has a higher modulus coefficient and tensile strength and less elongation than acidic films, and films prepared under alkaline conditions have a yellow color and an unpleasant taste [46]. The shelf life of the prepared gluten films is strongly dependent on the pH of the solution. During 120 days of storage, the hardness and Young coefficient in the acid film increased more than in the alkaline film.

The uniformity, thickness and smoothness of the surface remained at a higher level in alkaline films. The greater uniformity of alkaline films leads to an increase in oxygen inhibitory properties. Acidic gluten films showed greater volatility and migration of glycerol over storage time. Empty spaces in the acidic film are more than alkaline films [47].

*6.1.2.3. Ultrasound treatment*

The number and size of protein masses can be reduced by increasing the exposure time to ultrasound. Therefore, ultrasound treatment can be a useful physical strategy to obtain uniform gluten films under acidic conditions without the addition of chemical additives such as sodium disulfide [48].

*6.1.2.4. Heat treatment*

Heat has a compressive effect on wheat gluten, which causes the formation of disulfide bonds between molecules. As a result of heating at high temperatures, the strength and barrier properties of the film are improved. The temperature used should be higher than the glass transition temperature, but not too high to cause the protein to decompose. Protein-based materials are processed with 35-25% plasticizer, usually between 80 and 130 ° C.

The high process temperature limit can be increased by limiting or delaying the disulfide reaction. Salicylic acid is a natural product and is known as a radical scavenger. It also has no restrictions on use in packaging. Its antibacterial properties also maintain the quality of the protein during storage. It is very important that the film is compatible with existing technologies such as extruders [49].

*6.1.2.5. Mixing with hydrophobic materials*

Another way to improve the water vapor barrier properties of films is the participation of hydrophobic compounds such as lipids in the film-forming solution. The protein-lipid composite film is divided into two layers (in which the lipid is a separate layer between or above the protein film) and the emulsion (in which the lipid is uniformly distributed in the biopolymer film). If the lipid particles are small and evenly distributed, the water vapor permeability in the final film will be reduced. The most effective lipid in wheat gluten film to prevent moisture is wax. Mixing wheat gluten protein with di-acetyl tartaric ester monoglyceride reduces water permeability, increases tensile strength and maintains transparency [50].

*6.1.2.6. Nanocomposites*

A new group of expanded composites called nanocomposites, which has a reinforcing material less than or equal to 100 nanometer. In some studes, Montmorionite clay, SiO2, CuO and so have a lubricating effect and facilitates gluten injection molding. These nanoparticles increase the stiffness and strength of the molding sheets [51, 52].

*6.1.2.7. Impact of drying stage*

Drying stage is also an important part of the film formation process that can affect the final properties of the film. When drying, all volatiles are removed exponentially. Removal of the solvent increases the concentration of wheat gluten proteins, so the active components to form the bond are free and close enough to form new bonds to form a three-dimensional network with good oxygen barrier. The microstructure of the film depends on the rate at which the solvent evaporates. The rapid drop of the solvent causes the polymer to be less mobile and leads to a non-uniform distribution of gluten in the film. With increasing protein concentration, the films become stronger and show more tensile strength, the amount of elongation also decreases [53].

*6.2. Milk protein*

Milk protein, due to its high nutritional value and unique physical and chemical properties, is an important functional component in many processed foods. Types of protein-rich products such as casein and caseinate, whey protein concentrate (WPCs) and whey protein isolate (WPIs), milk protein concentrate (MPCs) is produced from milk protein. MPCs are produced directly from powdered milk with a combination of ultrafiltration/diafiltration. MPC can have a range of protein content from 56 to 82%. Casein in the micellar form is similar to that in milk, and cheese proteins are in their natural form. MPCs are used as additives in many food processes such as increasing the efficiency of cheese making, yogurt production and beverages. Milk proteins are good candidate to form biodegradable films [54].

6.2.1. Milk protein concentrate (MPC)

As the name implies, these products are obtained by concentrating the proteins in milk.

The main method of their production is ultrafiltration or diafiltration of after-wheel milk, as a result of which the proteins in milk are separated from water, lactose and other minerals in milk, and then by evaporation and spray drying processes, all kinds of powder products are produced. The protein content of this product varies from 35% to 80% and the basic protein of this product is casein and caseinate [55]. The most important features of this product can be mentioned as follows: high nutritional value (optimal amino acid profile), high amount of absorbable calcium, having a desirable taste, having a high water absorption, increasing the viscosity of products. The most important uses of this product can be mentioned as follows: tablets containing minerals and vitamins, instant drinks, a variety of nutritional foods (diet foods and sports drinks), a variety of pies, baby food, a variety of sauces , Ready meals, bakery products, confectionery and chocolate products [56].

*6.3. Gelatin*

Among biopolymers, gelatin is one of the best materials ever used to make films and edible coatings. Gelatin is a biopolymer derived from collagen. Gelatin is a colloidal animal protein and the oldest macromolecule obtained from the relative hydrolysis of insoluble collagen present in the bones and skin of animals and is able to produce gel during cooling. Collagen is the major component of connective tissue, making up the bulk of skin proteins, blood vessels, connective tissue, and bone and cartilage proteins [57, 58]. Gelatin is hydrophilic in nature and its gel resistance depends mainly on its concentration. It is used used in pharmacy to prepare capsule coatings. In the food industry, there have been reports of the antioxidant role when used as a coating in meat preservation [59].

Gelatin can produce films with desirable optical, mechanical and protective properties against gas, oxygen and odors at low relative humidity. Gelatin can be thought of as a copolymer consisting of soft blocks containing glycine, proline and alanine and hard blocks containing hydroxyproline, proline and glycine. The capacity of gelatin film formation and its efficiency as an outer layer of food to protect it against drying, light and oxygen have been extensively studied. Most gelatin films are not good protectors against water vapor due to their hydrophilic nature. Various ways to overcome these defects of gelatin films have been tested, including improving the mechanical properties by adding substances such as calcium, lactic acid and tannic acid. Modification of inhibitory properties is done by creating crosslinks, for which a variety of aldehydes can be used. Combining gelatin with other polymers and essential oils, as well as laminating and multilayering, greatly improves the performance of composite polymer films [60].

*6.4. Casein*

Casein is another protein substance used to make food films and coatings. Casein protein is the main protein in milk. This protein makes up 80% of cow's milk and the remaining 20% is Whey protein. The casein protein in milk was extracted by ultrafiltration and no chemicals were used. The result of this process is an increase in the amount of bioactive peptides in milk, which support immune functions and lead to muscle growth. Casein protein contains an amino acid and is known as a slow-digesting protein. There are three types of casein protein: calcium caseinate, micellar casein and milk isolate protein. Micellar casein is a pure type of casein and does not contain Whey protein. Isolated milk protein contains Whey and casein. In cow's milk, the isolated protein in milk is about 80 percent casein and 20 percent whey [61]. This protein is also found naturally in breast milk, but when consumed in excess from cow's milk in adulthood, it has completely different effects on the body. It is converted to casomorphin in the body, which has been reported to play a role in many diseases, including autism, apnea and pulmonary reflux in infants, type 1 diabetes, and various food allergies. In recent years, several articles have been reported on the addiction of casomorphins, which are found in dairy products, especially dairy cheese. It has been reported that the opioid potency of casomorphine is about 5% morphine, meaning that if taken in large amounts it can have comparable effects to morphine. Other reactions to casein include severe allergies that can occur in the form of skin allergies and anaphylactic and respiratory disorders [62].

## 7. Studies in the field of biodegradable protein polymers

Chavoshizadeh et al. (2020) studied wheat gluten/chlorophyll/polypyrrole (WG/Ch/PPy) nanocomposite film properties. According to their results, by adding chlorophyll and polypyrrole to gluten film, the solubility was significantly decreased. The opacity of films was significantly increased with adding chlorophyll and polypyrrole, where the effect of polypyrrole was higher than chlorophyll. With the addition of chlorophyll and polypyrrole, the antioxidant activity of the films was increased. According to the mechanical properties, the addition of chlorophyll and polypyrrole increased the tensile strength of the films. Also results showed that chlorophyll had no effect on the antibacterial property against Escherichia coli, but PPy enhanced the antibacterial character of gluten film significantly [63]. Fig. 5. Shows wheat gluten WG (A), wheat gluten/chlorophyll WG/Ch (B), wheat gluten polypyrrole WG/PPy (C) and wheat gluten/chlorophyll/polypyrrole WG/Ch/PPy (D) films [63].

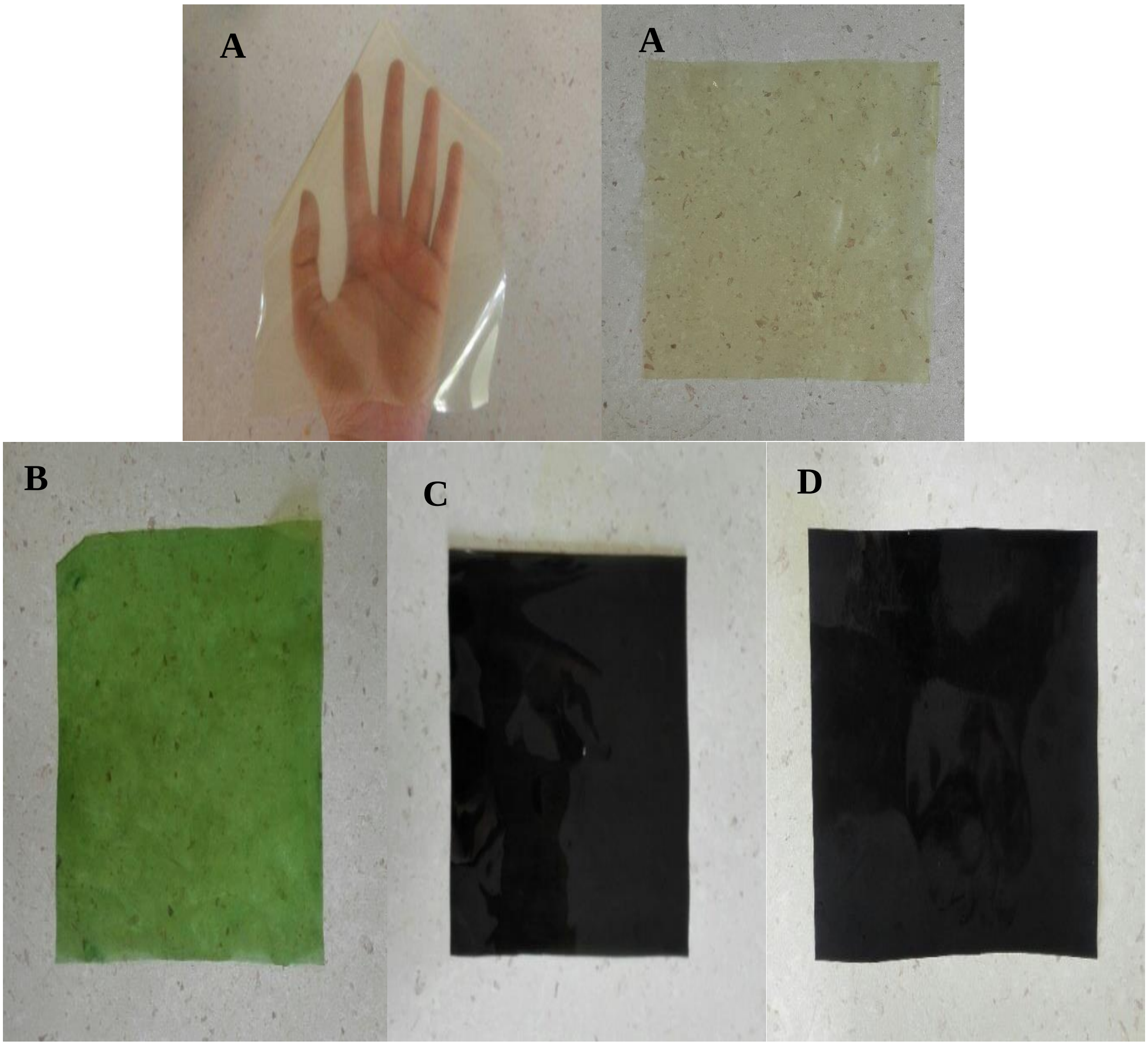


**Fig. 5.** WG (A), WG/Ch (B), WG/PPy (C) and WG/Ch/PPy (D) films

In 2014, Femandez-Pan et al. examined the effect of oral whey protein (WPI) protein coating containing clove and oregano essential oil on the quality properties of chicken fillets during refrigerated storage and observed that using the active coating, the shelf life of chicken meat increases from 6 to 13 days and the microbial load is significantly reduced [64]. Gimenez et al.

(2013) produced agar-gelatin composite film containing green tea extract. The results showed that the change in the concentration of agar and gelatin on the permeability to water vapor of the film is not effective. However, the solubility of the film increases with the increasing of concentration green tea extract. The films containing the extract showed strong antioxidant properties and antimicrobial properties [65]. Pirsa et al. (2020) synthesized Magnetic Gluten/Pectin/Fe3O4 Nano-hydrogel and studied its properties. Two types of composite Hydrogels including gluten/Fe3O4 and gluten/pectin/Fe3O4 composites were prepared by adding Fe3O4 nanoparticles and pectin to wheat gluten. The results confirmed the physical interactions between magnetic nanoparticles, pectin and gluten [66]. Rezaei et al. (2019) produced photocatalytic/antimicrobial active film based on wheat gluten/ZnO .anoparticles.

Their results about photocatalytic/antimicrobial activity of the films showed that pure gluten film had no effect on bacteria and fungi, but films containing zinc oxide nanoparticles showed a significant inhibitory effect on bacteria and fungi. The results of the antioxidant test showed that pure gluten had no antioxidant activity, but antioxidant activity increased with increasing zinc oxide nanoparticles significantly. The results of the FT-IR analysis showed that new interactions between zinc oxide nanoparticles and gluten polymer of wheat were created. The results showed that when the ZnO particle concentration was low, these particles were distributed uniformly, but high concentration of zinc oxide in the film leaded to agglomeration of these particles [67]. Farshchi et al. (2019) produced photocatalytic/biodegradable film based on carboxymethyl cellulose (CMC), modified by gelatin and TiO2-Ag nanoparticles. In this study FT-IR results showed that new interactions between the film components were created.

Scanning electron microscopy (SEM) results showed that the TiO2-Ag particles with 50–100 nm distributed in the CMC/Gelatin film. The results of the mechanical test showed that the TiO2-Ag nanoparticles at low concentrations increased tensile strength (TS) and decreased strain to break (STB), but with increasing nanoparticles concentrations, TS decreased and STB increased [68].

## 8. Conclussion

In recent decades, there has been great interest in the development of new technologies in the production of biodegradable films, which in addition to reducing dependence on fossil fuels and moving towards sustainable compounds, also has positive effects on the environment. However, the problem of using biopolymers in the production of films has some limitations, such as high permeability to moisture and poor mechanical properties, so many researchers are trying to improve the structure of biopolymers by adding different nanoparticles to strengthen their structure or the combination of several different biopolymers and the production of nanobiocomposites to improve the physical and mechanical properties of these compounds. Biodegradable protein films (gelatin, casein, wheat gluten, silk, wool, etc.) are the most important polymers used in the preparation of films and polymers. Protein films have good resistance to the passage of oxygen gas at low relative humidity and have good mechanical properties and turbidity, and therefore are more acceptable and used among biodegradable polymers. These films, like synthetic polymers, can be used in the packaging of many food products. These films can be modified with plant antioxidants such as essential oils and plant extracts or with antibacterial compounds such as silver, zinc oxide, copper oxide, etc. to form active polymers. The use of bioactive/antibacterial/antioxidant polymer films in food packaging can maintain the quality of the packaged product and increase its shelf life.

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